Decontamination of Beef Subprimal Cuts Intended for Blade Tenderization or Moisture Enhancement

C. E. HELLER,1 J. A. SCANGA,1,† J. N. SOFOS,1 K. E. BELK,1 W. WARREN-SERNA,2 G. R. BELLINGER,2 R. T. BACON,3 M. L. ROSSMAN,4 AND G. C. SMITH1

1Center for Red Meat Safety, Colorado State University, Fort Collins, Colorado 80523-1171; 2Food Safety Net Services, Ltd., 221 West Rhapsody, San Antonio, Texas 78216; 3McDonald's Corporation, 2111 McDonald’s Drive, Oak Brook, Illinois 60523; and 4National Cattlemen's Beef Association, 9110 E. Nichols Avenue, Centennial, Colorado 80112, USA

Abstract

The prevalence of Escherichia coli O157:H7 on beef subprimal cuts intended for mechanical tenderization was evaluated. This evaluation was followed by the assessment of five antimicrobial interventions at minimizing the risk of transferring E. coli O157:H7 to the interior of inoculated subprimal cuts during blade tenderization (BT) or moisture enhancement (ME). Prevalence of E. coli O157:H7 on 1,014 uninoculated beef subprimals collected from six packing facilities was 0.2%. Outside rot pieces inoculated with E. coli O157:H7 at 103 CFU/100 cm2 were treated with (i) no intervention, (ii) surface trimming, (iii) hot water (82°C), (iv) warm 2.5% lactic acid (55°C), (v) warm 5.0% lactic acid (55°C), or (vi) 2% activated lactoferrin followed by warm 5.0% lactic acid (55°C) and then submitted to BT or ME. Prevalence (n = 196) of internalized (BT and ME) E. coli O157:H7 was 99%. Enumeration of E. coli O157:H7 (n = 192) revealed mean surface reductions of 0.93 to 1.10 log CFU/100 cm2 for all antimicrobial interventions. E. coli O157:H7 was detected on 3 of the 76 internal BT samples and 73 of the 76 internal ME samples. Internal ME samples with no intervention had significantly higher mean E. coli O157:H7 populations than did those internal samples treated with an intervention, but there were no significant differences in E. coli O157:H7 populations among internal BT samples. Results of this study demonstrate that the incidence of E. coli O157:H7 on the surface of beef subprimal cuts is low and that interventions applied before mechanical tenderization can effectively reduce the transfer of low concentrations of E. coli O157:H7 to the interior of beef subprimal cuts.

Adding value to meat products has increasingly contributed to a steady and spectacular rise in per capita consumption of meat and poultry. Improvements in tenderness, flavor, juiciness, and water-holding capacity of meat products are desirable to consumers and have become a consumer-driven trend (7). Two types of mechanical treatments used to improve palatability of beef are blade tenderization and moisture enhancement via needle-injection.

In 1999, the U.S. Department of Agriculture Food Safety and Inspection Service expanded the Escherichia coli O157:H7 adulteration policy to include nonintact products (mechanically tenderized or restructured products) (12). However, they also acknowledged that pathogens can be translocated to the interior of mechanically tenderized products (5) and thus present a public health threat (1) if the product is not thoroughly cooked (71°C) (8).

The United States has experienced three E. coli O157:H7 outbreaks and two recalls associated with mechanically tenderized products. In 2000, illnesses related to E. coli O157:H7 associated with the consumption of mechanically tenderized products were reported in Michigan (18). In 2003, five illnesses prompted a voluntary recall of 335,506 kg (14) of frozen, raw, mechanically tenderized steaks. The following year, 184,545 kg of mechanically tenderized and ground beef products that may have been contaminated with E. coli O157:H7 were recalled (17); the recalled product was connected to six culture-confirmed illness cases and two probable cases in Minnesota with one confirmed case each in Michigan, Iowa, Kansas, and North Dakota (11).

The objectives of this study were to (i) determine the extent to which E. coli O157:H7 is present on the surface of subprimal cuts prior to mechanical tenderization in beef processing plants across the United States and (ii) evaluate the efficacy of five antimicrobial interventions applied 5 min before mechanical tenderization for minimizing the risk of transferring E. coli O157:H7 from the exterior to the interior of cuts during blade tenderization or moisture enhancement.

Materials and Methods

Uninoculated beef subprimal cuts, product sampling. A total of 1,014 beef cuts intended for blade tenderization (BT) or moisture enhancement (ME) were collected from six beef processing plants across the United States, including three purveying and three packing facilities, during a 5-week period in June and July. Samples were collected from a 200-cm2 surface area of each subprimal cut just before mechanical tenderization. Sampling was conducted with carcass sponges premoistened with buffered peptone water (BPW) and 100 cm2 sampling templates. Samples were sent in a shipping cooler with frozen gel packs via overnight courier to Food Safety Net Services, Ltd. (San Antonio, Tex.) for E. coli O157:H7 prevalence evaluation and enumeration.

† Author for correspondence. Tel: 970-491-6244; Fax: 970-491-5326; E-mail: john.scanga@colostate.edu.
Uninoculated beef subprimals, *E. coli* O157:H7 evaluation. Upon receipt, the sponge samples were massaged to ensure a homogenous mixture of BPW within the sponge. A 3-ml aliquot of the sample BPW was removed and stored in a sterile 15-ml conical tube at 4°C for future enumeration if required. Each of the samples was then enriched for 20 to 22 h at 37°C in tryptic soy broth (TSB; Becton Dickinson, Sparks, Md.) with 20 mg/liter novobiocin (Sigma, St. Louis, Mo.) and evaluated for the presence or absence of *E. coli* O157:H7 using the PCR-BAX system (Du-pong Qualicon, Wilmington, Del.) according to the manufacturer's written procedure. Samples that were positive for *E. coli* O157:H7 were processed for enumeration with the most-probable-number (MPN) procedure (15). Sample BPW aliquots stored at 4°C were diluted in triplicate in microtiter wells in a series to yield 1:10, 1:100, and 1:1,000 dilutions. Diluted samples were subjected to PCR-BAX to determine which samples contained *E. coli* O157:H7. These data were used to calculate the MPN of *E. coli* O157:H7 per square centimeter of the original sample, providing useful information in the determination of overall concentrations of this organism on subprimal beef cuts just before commercial mechanical tenderization.

Bacterial culture for inoculation of outside round pieces. Three strains of *E. coli* O157:H7 (ATCC 35150, FSNS 4312-4, and FSIS EC465-97) were combined in a cocktail that was prepared by streaking frozen stocks onto tryptic soy agar (Difco, Becton Dickinson) and incubating for 24 h at 35°C. Cultures were transferred to 100 ml of TSB and incubated an additional 18 to 24 h at 35°C. Liquid cultures were combined into a cocktail, centrifuged (Beckman Coulter GPR, Fullerton, Calif.) at 1,000 × g for 10 min, washed three times with Butterfield's phosphate buffer (BPB) (16), resuspended in fresh TSB, and diluted in BPB.

Inoculated outside round pieces, sample preparation and inoculation. Outside rounds were obtained from a commercial packing company over a 5-week period, the time required for experiments to be performed. Outside rounds were streaked with 10 ml of the three-strain *E. coli* O157:H7 cocktail to achieve an inoculation concentration of 10⁶ CFU/100 cm². Inoculation populations were determined by enumeration of surface samples taken immediately after inoculation, i.e., 12 100-cm² surface samples (three samples for each of four product shipments). Samples were spiral plated (Spiral Biotech Autoplate 4000, Advanced Instruments, Norwood, Mass.) onto MacConkey sorbitol agar (Becton Dickinson) supplemented with 0.05 µg/ml cefixime and 2.5 µg/ml tellurite (Dynal Biotech, Inc., Lake Success, N.Y.) (SMAC-ct) and incubated at 35°C for 24 h, and colonies were counted using an automatic plate counter (Advanced Instruments) running Q Count software (version 1.5, Spiral Biotech, Advanced Instruments); mean inoculation concentrations were 4.2 log CFU/100 cm². Inoculated outside rounds were individually vacuum packaged and stored for 10 to 18 days at 2 to 4°C.

Inoculated outside round pieces, intervention application. Samples for prevalence determination (*n* = 196) and enumeration (*n* = 192) were removed from storage and packaging and suspended from a sterilized meat hook. Either no treatment or one of five pathogen interventions was applied to the entire external surface of each outside round piece. Treatments groups were (i) no intervention (CT), (ii) surface trimming (ST) using good manufacturing practices (removal of surface fat with a sterile knife in approximately one single swipe), (iii) hot water (82°C) (HW), (iv) warm 2.5% lactic acid (LA) (55°C), (v) warm 5.0% LA (55°C), and (vi) 2% activated lactoferrin (AL) followed by warm 5.0% LA (55°C). The HW, 2.5% LA, 5% LA, and AL plus LA treatments were applied to outside round pieces with a handheld sprayer (RL FLO-MASTER, Root-Lowell Manufacturing, Lowell, Mich.) approximately 15.24 cm from the surface for 20 s at an approximate pressure of 3.1 bar. Outside round pieces were allowed a 5-min dwell time before undergoing BT or ME.

Inoculated outside round pieces, mechanical tenderization. Following storage, individual outside round pieces evaluated for the prevalence of *E. coli* O157:H7 (*n* = 196) were subjected to one of the five antimicrobial interventions and to either BT with a Honeywell blade tenderizer (Kansas City, Mo.) or ME with an Inject Star needle injector ("NT", "BI-52/72", Inject Star of the Americas, Inc., Brookfield, Conn.) and a solution of 0.5% sodium chloride (A.C. Legg, Inc., Calera, Ala.), 0.25% sodium tripolyphosphate (A.C. Legg), and 2.5% sodium lactate (Purac America, Lincolnshire, Ill.) (19). Outside round pieces were moisture enhanced such that they reached 112% of their green weight (64 strokes per min at 3.5 bar).

Following mechanical tenderization, all outside round pieces evaluated for prevalence of *E. coli* O157:H7 were suspended from a sanitized hook, and the entire external surface was seared with a propane torch to eliminate surface *E. coli* O157:H7 populations (prevent the transfer of surface inoculum to the internal surface during trimming). The external surface was then trimmed away using a sterile knife, the area where the hook had been inserted was cut off and discarded, and the remainder of each outside round piece was ground with a table top grinder (Univex, Salem, N.H.), placed in a Whirl-Pak bag (Nasco, Fort Atkinson, Wis.), and stored at 2 to 4°C for approximately 24 h. For each outside round piece, 25 g of ground product was enriched with 225 ml of TSB with novobiocin at a final concentration of 20 mg/liter and incubated at 37°C for 24 h. Enriched samples were assayed for *E. coli* O157:H7 using the PCR-BAX system. Between the mechanical tenderization of consecutive samples, all tables and cutting boards and the BT and ME machinery were rinsed with cold water and then sanitized with hot water (82 to 90°C), and the grinders were disassembled and thoroughly sanitized using soap and hot water (82 to 90°C).

Outside round pieces (*n* = 192) evaluated for *E. coli* O157:H7 by enumeration on SMAC-ct plates were mechanically tenderized with a tenderizer (model TC700M, Ross Industries, Inc., Midland, Va.) with a nominal production rate of 2,272 kg/h or with an Inject Star needle injector and a previously described brine solution (19) to 112% of the raw weight.

Samples for *E. coli* O157:H7 enumeration were collected with sterile knife at three points in the process: (i) surface samples (100 to 150 cm²) were excised from each outside round piece upon removal from vacuum packages (pretreatment intervention), (ii) following the application of interventions (posttreatment intervention), and (iii) following BT or ME (postprocessing). After BT or ME, round pieces were suspended from a sanitized hook, the inoculated surface was carefully removed with a knife, and the entire cut was seared with a propane torch to eliminate *E. coli* O157:H7 from the surface of the cut to prevent the transfer of surface inoculum to the internal surface while extracting sample. The segment of the subprimal cut where the hook had been inserted was removed and discarded, and the remainder of each piece, anterior surface up, was placed on a sanitized cutting surface. With a knife that had been sanitized (80°C for >1 min), a 5-cm-thick slice was removed from the approximate center (cuts were made perpendicular to the inoculated fat surface) of the remaining outside round piece and placed into a Whirl-Pak sample bag. Samples were transported to Food Safety Net Services, where they were removed from the sample bag. Each sample was split.
using a sterile scalpel and aseptic techniques into two 2.5-cm-thick slices perpendicular to the inoculated fat surface. A 100-cm² surface sample (<1.0 cm thick) was then excised from the surface of the newly exposed internal surface, such that a 200-cm² area sample was obtained when taking both surfaces into account. All results are reported per 100 cm².

Between mechanical tenderization of consecutive samples, all tables and cutting boards were rinsed with cold water at high pressure, scrubbed with soap and water, rinsed with cold water, sprayed with 5 ppm sodium hydrochlorite solution, and then rinsed with hot water (82 to 90°C). The BT and ME machinery was comparably sanitized, excluding the use of sodium hydrochlorite solution (which causes rusting and corrosion). Environmental samples were collected on each day of sampling from the BT machinery (one positive sample [day 5] of nine samples collected) and ME machinery (no positive samples of nine samples collected) for quality assurance. The risk of transferring *E. coli* O157:H7 to the interior of muscle samples is low and often dependent on site-specific cleaning and sanitation programs (3). Under unsanitary conditions and when there is a lack of awareness of bacterial recontamination, the tenderizer may become a bacterial inoculation machine (10).

For *E. coli* O157:H7 enumeration, the samples previously excised were shaved to obtain a subsample (100 cm², <1 cm thick) from the desired sampling surface. These subsamples were placed in a Whirl-Pak bag and weighed, and a volume of BPB equal to the weight of the subsample was added. The mixture was then pummeled with a stomacher (Seward, Norfolk, UK) for 1 to 2 min. A 0.1-ml aliquot of the mixture was spread plated at appropriate dilutions onto SMAC-ct and incubated at 37 ± 2°C for 24 to 26 h. Colonies morphologically typical of *E. coli* O157:H7 were counted.

**Uninoculated outside round pieces.** To more closely simulate conditions likely to be encountered in U.S. beef processing facilities, uninoculated outside rounds (*n* = 48) were subjected to antimicrobial interventions, mechanical tenderization, and sanitation measures as previously described (four samples per treatment).

**Statistical analysis.** Microbiological data (*E. coli* O157:H7 CFUs per 100 cm²) from preintervention, postintervention, and postprocessing samples were log transformed. Log reductions associated with interventions were determined by subtracting postintervention populations from preintervention populations. Results (CFUs per 1 cm²) from postprocessing samples were divided by the results from preintervention samples to determine the percentage of *E. coli* O157:H7 inoculum that was transferred from the external surface to the internal surface of outside round pieces (percent transference). These percentages were normalized by taking the square root of each value before analysis; nontransformed values are listed in the tables.

*E. coli* O157:H7 population data were analyzed using the general linear models procedure of SAS (Cary, N.C.), differences were determined using least-squares (LS) means with a significance level of 95% (*α* = 0.05). Reductions associated with treatment interventions were analyzed with the mixed models procedure of SAS, and differences between treatment interventions were determined using LS means with a significance level of 95%. Degrees of freedom were calculated according to the Satterthwaite approximation. Independent variables in the mixed models procedure were intervention, mechanical tenderization technique (BT or ME), and the interaction of intervention × mechanical tenderization technique; random variables were inoculation group and replicate.

The percentage of *E. coli* O157:H7 inoculum transferred from the external to the internal surface of the cut was analyzed using the mixed models procedure of SAS. Differences were determined using LS means with a significance level of 95%, and degrees of freedom were calculated using the containment method. Independent variables in the mixed models procedures were intervention, mechanical tenderization technique (BT or ME), and the interaction of intervention × mechanical tenderization technique; random variables were inoculation group and replicate.

A SAS chi-square goodness-of-fit analysis was conducted to verify that similarities (*P* < 0.05) and differences (*P* < 0.05) among treatment interventions were not attributable to the number of samples with *E. coli* O157:H7 numbers below the detection limits of SMAC-ct enumeration.

**RESULTS AND DISCUSSION**

**Uninoculated beef subprimal cuts.** Of the 1,014 samples evaluated over the 5-week test period, 2 (0.2%) were positive for *E. coli* O157:H7 with the PCR-BAX method. These samples were subjected to MPN analysis because this method has been useful for enumeration of organisms found in low concentrations (2). For these two positive samples, serial dilutions were made from the original sample aliquots maintained at 4°C, and the MPN analysis revealed that each positive sample had *E. coli* O157:H7 concentrations of <0.375 CFU/cm². In an adjusted prevalence analysis of *E. coli* O157:H7 in ground beef based on the proportion of carcasses that become primary cuts of meat, rather than trim, an estimated 0.02% of steaks produced annually are expected to contain one *E. coli* O157:H7 cell (13). Results from both analyses indicate that *E. coli* O157:H7 is not commonly found on the surface of subprimal beef cuts. Thus, internal contamination of subprimal cuts by *E. coli* O157:H7 via mechanical tenderization is unlikely to occur but still is possible, as indicated by the 2000 (18), 2003 (14), and 2004 (17) *E. coli* O157:H7 outbreaks associated with nonintact beef products.

**Inoculated outside round pieces, prevalence.** The prevalence of *E. coli* O157:H7 within outside round pieces that had been inoculated with an *E. coli* O157:H7 cocktail at 4.2 log CFU/100 cm², subjected to one of four antimicrobial interventions, and further processed by BT or ME was 99% (194 of 196 samples). Two samples, one ME sample that had been sanitized with hot water (82°C) and one BT sample that had been sanitized with warm 2.5% LA, did not test positive by the PCR-BAX method (Table 1). Prevalence results were not useful in determining differences among treatment groups; therefore, enumeration on SMAC-ct plates was incorporated into the study. These results of high internal prevalence were attributed to the fact that the inoculation concentrations used in this study were far higher than the concentrations of *E. coli* O157:H7 one would expect to find on uninoculated beef surfaces (0.2% of cuts, <0.375 CFU/cm²; 392 times lower than the inoculation concentration).

**Inoculated outside round pieces, enumeration.** After refrigerated anaerobic storage, surface samples were collected before the application of treatment interventions or mechanical tenderization (preintervention). The mean pop-
TABLE 1. Prevalence of E. coli O157:H7, as determined by PCR-BAX, after inoculated outside beef round pieces were subjected to one of four antimicrobial interventions and either blade tenderization (BT) or moisture enhancement (ME).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>BT</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive controlb</td>
<td>26/26 (100)</td>
<td>24/24 (100)</td>
</tr>
<tr>
<td>Trimmingc</td>
<td>13/13 (100)</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>Hot waterd</td>
<td>13/13 (100)</td>
<td>11/12 (91.7)</td>
</tr>
<tr>
<td>Warm 2.5% LAc</td>
<td>11/12 (91.7)</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>Warm 5% LAd</td>
<td>24/24 (100)</td>
<td>24/24 (100)</td>
</tr>
<tr>
<td>AL + warm 5% LAe</td>
<td>12/12 (100)</td>
<td>12/12 (100)</td>
</tr>
</tbody>
</table>

Values are least-squares means.

a Values are the no. of positive samples/no. of samples tested (% of positive samples).
b Positive control samples were inoculated but not subjected to an antimicrobial intervention.
c Samples were carefully trimmed to remove all external surfaces before further processing.
d Hot water (82°C) was sprayed onto the surface of each cut for 20 s.
e Warm 2.5% lactic acid (LA) (55°C) was sprayed onto the surface of each cut for 20 s.
f Warm 5.0% LA was sprayed onto the surface of each cut for 20 s.
g% activated lactoferrin (AL) solution was sprayed onto the surface of each cut for 20 s, followed by a spray application of warm 5.0% LA for 20 s.

TABLE 2. E. coli O157:H7 population collected from the surface of inoculated outside round pieces before (PRE) and after (POST) antimicrobial intervention, the population reduction due to the intervention (RED), and the percent survival (SUR) after intervention.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>PRE</th>
<th>POST</th>
<th>RED</th>
<th>% SURb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive controloc</td>
<td>3.5 ± 0.07</td>
<td>A</td>
<td>2.6 ± 0.07 A</td>
<td>1.1 ± 0.07 c</td>
</tr>
<tr>
<td>Trimmingd</td>
<td>3.7 ± 0.07 A</td>
<td>2.6 ± 0.07 A</td>
<td>1.0 ± 0.07 c</td>
<td>72.0</td>
</tr>
<tr>
<td>Hot watere</td>
<td>3.6 ± 0.07 A</td>
<td>2.6 ± 0.07 A</td>
<td>1.0 ± 0.07 c</td>
<td>72.2</td>
</tr>
<tr>
<td>Warm 2.5% LAc</td>
<td>3.6 ± 0.07 A</td>
<td>2.6 ± 0.07 A</td>
<td>1.0 ± 0.07 c</td>
<td>72.2</td>
</tr>
<tr>
<td>Warm 5% LAd</td>
<td>3.5 ± 0.07 A</td>
<td>2.4 ± 0.07 A</td>
<td>1.1 ± 0.07 c</td>
<td>68.5</td>
</tr>
<tr>
<td>AL + warm 5% LAh</td>
<td>3.5 ± 0.07 A</td>
<td>2.6 ± 0.07 A</td>
<td>0.9 ± 0.07 c</td>
<td>74.2</td>
</tr>
</tbody>
</table>

Values are least-squares means ± standard errors. Within rows and columns, means lacking common letters are significantly different (P < 0.05).
b Percentage of inoculum (log CFU/100 cm²) recovered from outside round surface following intervention (PRE/POST).
c Positive control samples were inoculated but not subjected to an antimicrobial intervention.
d Samples were carefully trimmed to remove all external surfaces before further processing.
e Hot water (82°C) was sprayed onto the surface of each cut for 20 s.
f Warm 2.5% lactic acid (LA) (55°C) was sprayed onto the surface of each cut for 20 s.
g Warm 5.0% LA was sprayed onto the surface of each cut for 20 s.
h A 2% activated lactoferrin (AL) solution was sprayed onto the surface of each cut for 20 s, followed by a spray application of warm 5.0% LA for 20 s.

Inoculated outside round pieces, internal enumeration. Internal surface samples from outside round pieces subjected to BT had lower E. coli O157:H7 populations (P < 0.05) than did either preintervention or postintervention surface samples. Three of the 76 samples subjected to an intervention had detectable E. coli O157:H7; mean internal E. coli O157:H7 counts for those treatment interventions were below, equal to, or slightly higher than detectable limits of the analysis (10 CFU/100 cm² is the detection limit for SMAC-cf, which was equivalent to 5 CFU/200 cm² for postprocessing samples; Table 3). BT of CT samples resulted in a mean transfer of 0.41% CFU/cm² (0.75 log CFU/100 cm²; Table 4) of the preintervention inoculation population (3.4 log CFU/100 cm²; Table 3). Transfer of surface inoculum ranged from 0.28 to 2.74%, a lower and broader range than the 3 to 4% reported in another study for beef samples inoculated with 10³ and 10⁶ CFU/cm² (9) and a smaller and narrower range than the 1 to 7% of inoculum (10⁵ and 10⁶ CFU/cm²) transferred to the internal portions of pork cuts (6). Following ME, mean internal E. coli O157:H7 counts were 1.82, 1.02, 0.93, 1.11, 0.82, and 1.08 log CFU/100 cm² for CT, TR, HW, 2.5% LA, 5.0% LA, and AL + LA (Table 5), respectively. Internal outside round samples submitted to ME had (P < 0.05) E. coli O157:H7 counts that were lower than either preintervention or postintervention surface counts. For CT samples, ME resulted in a mean transfer of 1.94% (Table 4) (1.82 log CFU/100 cm²; Table 5) of the preintervention E. coli O157:H7 population of 3.6 log CFU/100 cm². Transfer ranged from 0.30 to 3.09%, a
TABLE 3. E. coli O157:H7 population recovered from the external surface of inoculated outside round pieces before (PRE) and after (POST) antimicrobial intervention and from the internal surface following blade tenderization (BT)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>E. coli O157:H7 (log (CFU/100 cm²))</th>
<th>No. positive&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.4 ± 0.09 A</td>
<td>0.75 ± 0.05 A</td>
</tr>
<tr>
<td>Trimming&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.6 ± 0.09 A</td>
<td>2.5 ± 0.09 B</td>
</tr>
<tr>
<td>Hot water&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.6 ± 0.09 A</td>
<td>2.6 ± 0.09 B</td>
</tr>
<tr>
<td>Warm 2.5% LA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.6 ± 0.09 A</td>
<td>2.6 ± 0.09 B</td>
</tr>
<tr>
<td>Warm 5% LA&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3.4 ± 0.09 A</td>
<td>2.4 ± 0.09 B</td>
</tr>
<tr>
<td>AL + warm 5% LA&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.5 ± 0.09 A</td>
<td>2.5 ± 0.09 B</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are least-squares means ± standard errors. Within rows and columns, means lacking common letters are significantly different ($P < 0.05$). Detection limits: 10 CFU/100 cm² (1 log CFU/100 cm²) for PRE and POST and 5 CFU/100 cm² (0.699 log CFU/100 cm²) for BT.

<sup>b</sup> Of the 16 BT samples tested for each intervention, the no. of samples with detectable E. coli O157:H7 is given.

<sup>c</sup> Positive control samples were inoculated but not subjected to an antimicrobial intervention.

<sup>d</sup> Samples were carefully trimmed to remove all external surfaces before further processing.

<sup>e</sup> Hot water (82°C) was sprayed onto the surface of each cut for 20 s.

<sup>f</sup> Warm 2.5% lactic acid (LA) (55°C) was sprayed onto the surface of each cut for 20 s.

<sup>g</sup> Warm 5.0% LA was sprayed onto the surface of each cut for 20 s, followed by a spray application of warm 5.0% LA for 20 s.

<sup>h</sup> 2% activated lactoferrin (AL) solution was sprayed onto the surface of each cut for 20 s.

lower and narrower range than the 4 to 8% reported for pork samples inoculated with 10<sup>5</sup> and 10<sup>6</sup> CFU/cm² (6).

All ME samples treated with an intervention transferred less than 1.05% CFU/cm² of surface E. coli O157: H7 to the internal portion of the outside-round piece, with an overall mean transfer level of 0.35% CFU/cm². When comparing treatment interventions for ME samples, AL plus LA was as effective as 2.5% LA and 5% LA for surface reductions, though it was less effective than 5% LA relative to the percentage of surface inoculum that was transferred to the internal surface of ME outside round pieces. LA plus AL had the highest mean transfer percentage at 0.46% CFU/cm² (Table 4), 1.08 log CFU/100 cm² (Table 5), significantly higher ($P < 0.05$) than 5% LA, which had the lowest transfer percentage at 0.25% (Table 4), 0.82 log CFU/100 cm² (Table 5).

E. coli O157:H7 transfer from ME samples treated with no intervention (CT) was 4.7 times greater ($P < 0.05$) than that for BT samples, suggesting that ME more efficiently internalized surface bacteria than did BT, especially when high numbers of microorganisms are present. It has been suggested that the design of tenderizing machines and blades can affect internalization rates; machinery with parts easily accessible for cleaning and thin blades designed to minimize the number of bacteria carried beneath the incised surface may result in reduced pathogen transfer (4).

E. coli O157:H7 was found in nearly all samples based on prevalence analyses, although enumeration results did not agree, particularly for internal BT samples. The difference in prevalence and enumeration results can be attributed to the enrichment step utilized in the prevalence protocol to increase the sensitivity of the PCR-BAX analysis. Samples that were processed for enumeration were not enriched to allow determination of the actual population of E. coli O157:H7; therefore, the samples reported as having populations below the detection limit of the SMAC-ct procedure (<10 CFU/100 cm²) would have returned a positive PCR-BAX test following enrichment.

Uninoculated outside round pieces. Before antimicrobial interventions were applied, 46 of 48 uninoculated outside rounds (22 subjected to BT and 24 subjected to...
ME) had undetectable *E. coli* O157:H7. The two uninoculated samples positive for *E. coli* O157:H7 may have been cross-contaminated while in the research facility, although extensive measures were taken to sterilize all work surfaces and equipment. Following application of one of five antimicrobial interventions, none of the subsequent external or internal surface samples had detectable *E. coli* O157:H7.

The use of mechanical tenderization practices improves the marketability of certain beef cuts. However, there is an inherent albeit low risk of contracting foodborne illnesses when consuming undercooked mechanically tenderized beef products. BT and ME resulted in the transfer of pathogen to the interior of inoculated cuts, although transfer rates were higher for ME samples than for BT samples. Application of surface trimming, hot water (82°C), warm 2.5% LA (55°C), warm 5% LA, or 2% AL followed by warm 5% LA reduced pathogen loads on the surface of inoculated subprimal cuts, thus subsequently reducing the internalization of surface pathogens. The application of an antimicrobial intervention before mechanical tenderization will reduce low levels of contamination to nondetectable levels, reducing the risk of encountering foodborne illness from nonintact blade-tenderized or needle-injected or enhanced beef products.

**ACKNOWLEDGMENTS**

This research was funded in part by beef and veal producers and importers through their $1-per-head checkoff (Cattlemen’s Beef Board) and by the American Meat Institute Foundation.

**REFERENCES**


